Correlations between thyroidal and reproductive endocrine status in wild Atlantic cod

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ABSTRACT

We monitored thyroidal and reproductive hormones in blood serum from wild Atlantic cod (*Gadus morhua* L.) over a two-year period. Here we examine potential interactions between the two hormone groups. We found few indications of interactions in males. However, rising levels of L-thyroxine (T_4) and 3,5,3'-triiodo-L-thyronine (T_3) in females concurred with early ovarian development. T_3 levels declined after the ovaries reached a certain size (gonadosomatic index *c.* 3.0) and after estradiol-17β (E_2) rose above 1.0 ng ml^{-1}. We suggest the thyroidal and reproductive systems in cod interact during certain phases of the ovarian cycle.

RÉSUMÉ

Nous avons effectué un suivi des hormones thyroïdiennes et gonadiques dans le sérum de la morue (*Gadus morhua* L.) pendant une période de deux ans. Dans ce document, nous examinons les interactions potentielles entre les deux groupes d'hormones. Nous avons trouvé peu d'indication d'une interaction potentielle chez les individus males. Toutefois, chez les individus femelles, une augmentation des niveaux de L-thyroxine (T_4) et tri-iodo-L-thyronine (T_3) coïncida avec le début du développement ovarien. Les niveaux de T_3 diminuèrent suite à une certaine croissance des ovaires (indice gonadosomatique d'environ 3,0) et lorsque les niveaux d'estradiol augmentèrent à des niveaux au-delà de 1,0 ng ml^{-1}. Nous suggérons que les systèmes thyroïdiens et reproducteurs chez la morue interagissent pendant certaines phases de cycle ovarien.

INTRODUCTION

There is mounting evidence that complex interactions between the thyroidal and reproductive endocrine systems provide some control over competing somatic and gonadal growth processes in salmonid and freshwater teleosts (see Dickhoff *et al.*, 1989; Cyr & Eales, 1996). However, little is known about the two endocrine systems in marine fish. Woodhead (1959, 1975) argued that major seasonal changes in thyroidal activity in the Barents Sea cod *Gadus morhua* L. were more closely related to migratory behaviour than to reproductive function. Since then the course of thyroid hormones (TH) over at least one complete reproductive cycle has been documented for plaice *Pleuronectes platessa* L. (Osborn & Simpson, 1978), winter flounder *Pseudopleuronectes americanus* Walbaum (Eales & Fletcher, 1982), and more recently cod (Comeau *et al.*, 2000). No significant correlations between thyroidal and reproductive status were found (plaice, flounder) or reported (cod).

Possible reasons for the difficulty in detecting links between the two endocrine systems include the criteria used for the assessment of endocrine status. The thyroid histological indices used in the study of Barents Sea cod (Woodhead 1959, 1975) are now considered unreliable for detecting short-term changes in the circulating levels of thyroid hormones (Eales & Brown, 1993; Cyr & Eales, 1996). Similarly, because only total (protein bound and unbound) TH levels were measured in pleuronectiformes (Osborn & Simpson, 1978; Eales & Fletcher, 1982), certain interactions between the reproductive and peripheral thyroid systems may have gone undetected (Cyr & Eales 1989, 1992). Moreover, TH levels in pleuronectiformes were compared with gonadal condition indices, which may not have been reflective of gonadal hormone levels (e.g., see Pankhurst & Conroy, 1987; Pankhurst & Kime, 1991; Methven *et al.*, 1992; Kjesbu *et al.*, 1996).

Our main objective was to correlate the thyroidal and reproductive systems in wild cod. We closely monitored the natural course of endocrine hormones, including E_2 and free 3,5,3'-triiodo-L-thyronine (T_3), over two complete reproductive cycles. Our results led to the conclusion that interactions between the two endocrine systems in this marine teleost are plausible, particularly in females. We discuss the biological implications arising from these results.

MATERIALS AND METHODS

SAMPLE ATTRIBUTES

Sexually matured Atlantic cod were sampled from the southern Gulf of St. Lawrence population between July 1995 and November 1997. The number of fish collected each month ranged from 13 to 224 (average = 91) and the two sexes were generally well represented. The mean fork length of cod in samples varied from 47 to 57 cm (average = 52 cm, SE = 0.5 cm). Most samples were captured using mobile gear vessels that carried out 60 min sets. In October and November 1996, however, 43% of the samples were collected using baited-longlines. The soaking time of longlines ranged from 4 to 24 h. No effects of gear-type on the concentrations of hormones were found (Mann-Whitney test, $P < 0.22$) when comparing samples collected with mobile and longline gear in the same area on the same day.

Blood samples were retrieved within about 20 minutes after the fish were boated. The aorta was cut and blood was allowed to flow directly into 7-ml Vacutainer vials. The vials were capped and refrigerated for 12 to 24 h. The serum fraction was then transferred into 2.5-ml cryovial tubes and stored at -72° C until analysed. Cod were kept on ice and dissected within 48 h or frozen at -20° C and dissected after having been partially thawed. We determined the gonadosomatic index (I_G) as 100 × the wet weight of the gonads divided by the wet weight of the carcass (total body weight minus combined weight of liver, gonads, and stomach) (Schwalme & Chouinard, 1999).

HORMONE MEASUREMENTS

We measured total L-thyroxine (T_4) and total T_3 on 1,665 serum samples using a combined radioimmunoassay (RIA) technique (Omeljianuk *et al.*, 1984) and the reagents employed by Cyr *et al.* (1998). Details of the procedures are also given in Comeau *et al.* (2000). Of the 1,665 samples, 856 contained a sufficient amount of serum to determine the fraction of T_3 that was freed from carrier proteins. A free T_3 index, representing the percentage of serum T_3 in the free form, was measured on G-25 Quik-Sep columns (Isolab Inc., Akron, OH) according to the procedure of Eales & Shostak (1985) adapted for cod serum (Comeau *et al.*, 2002). The free T_3 index was converted into concentration values using total T_3 results from the combined T_4-T_3 RIA and the following formula:

$$\text{free } T_3 \text{ [ng ml}^{-1}\text{]} = \text{total } T_3 \text{ [ng ml}^{-1}\text{]} \times \text{free } T_3 \text{ index [\%]} \times 10^{-2}$$

Gonadal hormones were measured using commercial Coat-A-Count® RIA kits (Diagnostic Products Corporation, Los Angeles, CA). A total of 1,115 samples (429 males, 686 females) were processed for the measurement of E_2 levels. A similar number of samples (569 males, 530 females) were analysed for testosterone levels.

Radioactivity was measured on duplicate samples using a Beckman Gamma 5500 Counting System. Except for free T_3 (not applicable), the dilution of serum samples yielded hormone levels close to the predicted values, and the dilution curves of samples were parallel to the dilution curves of standards. Intra- and interassay coefficients of variation were less than 10%.

STATISTICS

Monthly means of TH and the I_G were plotted against time. To examine the thyroidal profiles as a function of reproductive status, we ranked the reproductive variable (I_G, E_2, or testosterone) based on percentile groups; 25 groups were created, with each group containing approximately 36 cases (individuals). Arithmetic means of reproductive and thyroidal measurements were computed for individual groups; the means were plotted against each other and visually inspected for trends.

Simple and multiple linear regression analyses were used to investigate the relationships between the free T_3 index and three explanatory variables (E_2, testosterone, total T_3). The analyses were based on individual fish and were restricted to narrow phases of the reproductive cycle, each extending over a three-month period. For females, the free T_3 index was $\log_{10}$ transformed to ensure linearity; for males, the total T_3 variable was $\log_{10}$ transformed. In multiple regression analysis, we tested the following models for females and males, respectively:

$$\log_{10} \text{free } T_3 \text{ index} = \beta_0 + \beta_1 \cdot E_2 + \beta_2 \cdot \text{testosterone} + \beta_3 \cdot \text{total } T_3$$

$$\text{free } T_3 \text{ index} = \beta_0 + \beta_1 \cdot E_2 + \beta_2 \cdot \text{testosterone} + \beta_3 \cdot \log_{10} \text{total } T_3$$

where β_0 represents the intercept and $\beta_{1\rightarrow3}$ are the slopes. The entry and removal of independent variables from the models was based on a stepwise selection approach; the *P*-to-enter and *P*-to-remove values were set at 0.05 and 0.10, respectively. Residual plots and the Durbin-Watson test indicated that the error terms met the assumptions of linear regression analysis.

RESULTS

TH levels generally increased during gonadal recrudescence in autumn (Figure 1). The magnitude of the T_4 upsurge, however, was much higher—by 85% on average—in females than in males ($P < 0.01$, t-test for paired comparisons applied to November – March monthly means). Also, the timing of the upsurge was better synchronized with early ovarian growth (I_G *c.* 1.0 → 3.0, Figure 2a) than with early testes growth.

TH levels started declining abruptly during late ovarian development (I_G *c.* 3.0, Figure 2a). In males, T_3 levels declined (slightly) when the testes reached a certain size (I_G *c.* 2.0, Figure 2b). Testosterone and E_2 levels were elevated during late gonadal development (Figure 2c, d). However, total T_4 levels presented no trend in relation to rising gonadal hormone levels (Figure 2e, f). Only total T_3 levels in females clearly started falling as E_2 levels rose above 1.0 ng ml^{-1} (Figure 2e).

The free T_3 index presented two major seasonal shifts (Figure 3a): it declined significantly in females in winter (autumn *v.* winter, $P < 0.01$, Mann-Whitney test), and increased in both sexes in spring (winter *v.* spring, $P < 0.001$, Mann-Whitney test). These seasonal changes are noteworthy because they rendered the absolute free T_3 *levels* less variable across seasons (Figure 3b). For instance, modelling work indicates that mean free T_3 levels in females would have risen to 1.8 ng ml^{-1} (SE = 0.2) in winter had the free T_3 index been fixed to the autumnal values; in reality, mean free T_3 levels in females peaked at 1.3 ng ml^{-1} (SE = 0.2) in winter. By shifting its course again in spring, the free T_3 index upheld free T_3 levels close to 0.6 ng ml^{-1} (SE = 0.1) in both sexes. Without this second shift in the free T_3 index, modelling work shows that free T_3 levels would have declined to values near 0.36 ng ml^{-1} (SE = 0.04) in spring.

We found negative correlations between gonadal hormones and the free T_3 index in females in winter (Figure 4). E_2 and testosterone respectively explained 51% ($P < 0.001$) and 60% ($P < 0.001$) of the variability associated with the free T_3 index between the months of November and January. Testosterone was retained as the strongest predictor in a multiple regression model that explained 68% ($P < 0.001$) of the variability in free T_3 (Table 1). In spring, however, total T_3 became the best predictor of changes in free T_3 (Figure 5; Table 1). According to the models, the tendency to free T_3 from plasma proteins in spring was due in large part to low T_3 substrate availability. Also, the relationships were significant only for cases with total T_3 levels below 35 ng ml^{-1}, indicating a possible threshold for triggering the release of T_3 from binding proteins.

DISCUSSION

EFFECTS OF THYROID HORMONES ON REPRODUCTIVE FUNCTION

Woodhead (1959, 1975) suggested that enhanced thyroidal activity in cod is functionally related to migratory behaviour. In the present study, we report that TH may also aid in the initiation of new reproductive cycles. We found appreciable total T_3 levels during testicular recrudescence, consistent with enhanced T_3 substrate in the testes of chum salmon *Oncorhynchus keta* Walbaum (Tagawa *et al.*, 1994), and also with the presence of nuclear T_3 receptors in the testes of freshwater perch *Anabas testudineus* Bloch (Jana & Bhattachcharya, 1993). In cod, the thyroidal upsurge in relation to ovarian recrudescence was particularly remarkable and clearly involved both T_3 and T_4. The exact significance of such endocrine activity is not known, although previous work suggest that TH enhance vitellogenin uptake from plasma into the eggs (Shibata *et al.*, 1993) and act in synergy with gonadotrophins to stimulate early ovarian follicle growth (Cyr & Eales, 1988, 1996). The thyroidal upsurge in female cod may thus have been related to early oocyte maturation.

EFFECTS OF GONADAL HORMONES ON THYROIDAL FUNCTION

Previous work on salmonids suggested that rising E_2 levels during the later stages of ovarian development exert a negative effect on the thyroid gland, thereby reducing TH levels in circulation (Leatherland, 1985; Yamada *et al.*, 1993). Falling TH levels are likely required for the final maturation of the ovaries. In cod, we found a close association between late ovarian growth ($I_G > 3.0$) and falling total T_4 levels, although there was no evidence that this decline in T_4 was driven specifically by E_2. On the other hand, our data are in agreement with the premise that E_2 suppresses the peripheral thyroid status, namely T_3 production rates (Leatherland, 1985; Cyr *et al.*, 1988; Flett & Leatherland, 1989; Yamada *et al.*, 1993) and the free T_3 index (Cyr & Eales, 1989).

Testosterone might have also contributed to the lowering of the free T_3 index. Both steroids were present in appreciable concentrations in females, and their combined effect provides a reasonable explanation for the free T_3 index declining significantly in females in winter.

Falling (< 35 ng ml^{-1}) total T_3 levels, however, seemed largely responsible for the elevation of the free T_3 index in spring. This finding is consistent with the observation that arctic charr *Salvelinus alpinus* L. with low total T_3 levels contained relatively few T_3 binding proteins (Eales & Shostak, 1985). In cod, as in many other teleosts, the liver manufactures the bulk of the circulating T_3 (Eales & Brown, 1993; Cyr *et al.*, 1998). Poor feeding conditions on winter grounds (Schwalme & Chouinard, 1999) likely depressed hepatic T_3 production rates in southern Gulf cod (Comeau *et al.*, 2000), perhaps triggering in turn a reduction in the number of T_3 binding proteins. We conclude that gonadal hormones are probably not the only factors influencing the free T_3 index in wild cod, especially at times when T_3 production rates fall below a certain threshold.

BIOLOGICAL IMPLICATIONS

In cod, it is likely that the release of T_4 by the thyroid is activated at the autumn equinox (Comeau *et al.*, 2001). Therefore, endocrine stimulation of ovarian recrudescence may be synchronized in several cod populations throughout the northern hemisphere. To date, evidence of ovarian cycles being initiated near the autumnal equinox is available for southern Gulf of St. Lawrence cod (Schwalme & Chouinard, 1999), northern Gulf of St. Lawrence cod (Lambert & Dutil, 1997), Arctic cod (Hop *et al.*, 1995), Barents Sea cod (Woodhead & Woodhead, 1965; Ponomarenko, 1996), and Pacific cod (Smith *et al.*, 1990).

Once ovarian growth is under way, continued interactions between the two endocrine systems may allow some control over competing somatic and gonadal growth processes. More precisely, it is known that the free T_3 fraction is metabolically potent since it can readily move into target cells and interact with nuclear receptor sites (Eales & Brown, 1993). It is also known that T_3 is more effective than T_4 in promoting fish growth (Higgs *et al.*, 1979). Accordingly, in the female cod, the curtailment of free T_3 levels in winter likely restricts the metabolism and somatic growth rates, and in doing so may allow the metabolic reserves to be directed towards continued oocyte development (Cyr & Eales, 1996). During the spring period, there are probably some adaptive advantages in upholding free T_3 levels above a certain threshold. This second peripheral endocrine adjustment, which was unexpected and poorly correlated with reproductive status, may be necessary for sustaining cell metabolism during a lengthy (500 km – Hanson, 1996) spawning migration in spring.

CONCLUSION

We found that interactions between the thyroidal and reproductive systems in cod were plausible for certain phases of the reproductive cycle, particularly in females. It is suggested that TH contributed to the stimulation of ovarian recrudescence, and that rising levels of E_2 and testosterone, both present in females, exerted a negative effect on the peripheral thyroid status. Despite major differences in reproductive strategies, similar interactions were reported for salmonids (Cyr & Eales, 1996). Thus, it appears that the interactions between the two endocrine systems are important regulators of basic physiological processes such as growth and reproduction.

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Table 1. Results of stepwise multiple regression analyses with the free T_3 index as the dependent variable. The *t* statistics provides an indication of the relative importance of each parameter; the *P* and Probability values indicate the significance of individual parameter and of the overall regression, respectively.

	E_2		Testosterone		Total T_3		Final Model	
Period	t	*P*	t	*P*	t	*P*	r^2	Probability
Nov – Jan (♀)	-1.63	0.11†	-8.94	<0.001	-2.82	<0.01	0.68	<0.001
Jan – Mar (♀)	0.37	0.72†	-3.43	<0.01	-4.14	<0.001	0.58	<0.001
(♂)	0.88	0.39†	-0.36	0.73†	-6.71	<0.001	0.58	<0.001

† parameter excluded from model

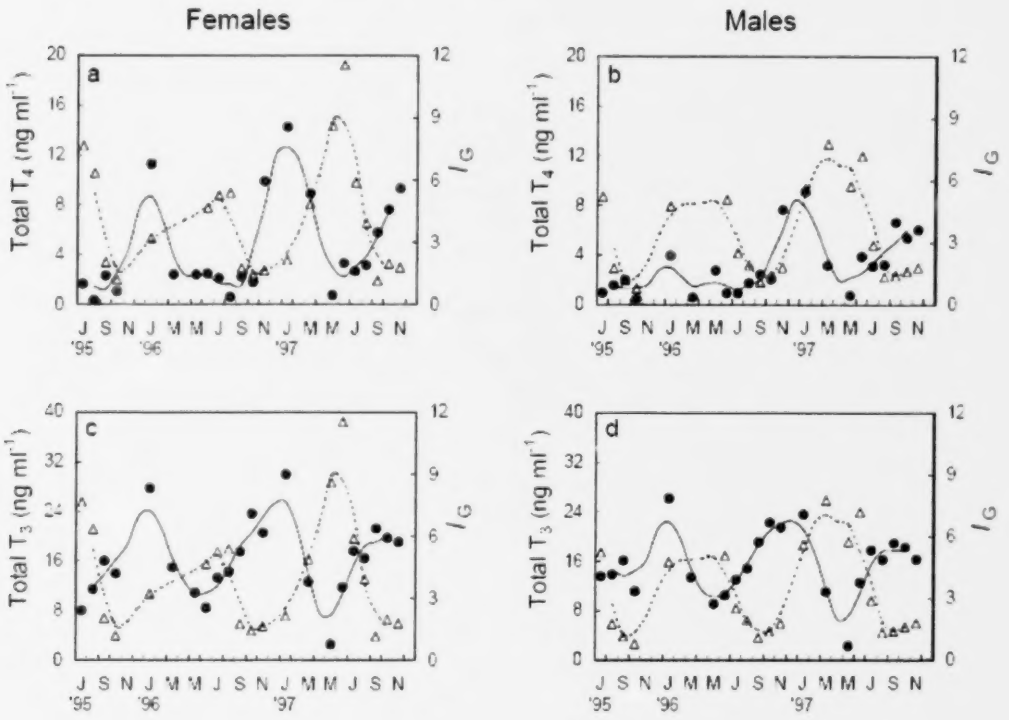

Figure 1. Seasonal changes of total T_4, total T_3 and I_G. ●, monthly means of either total T_4 [(a), (b)] or total T_3 [(b), (c)]; Δ, monthly means of I_G. Trend lines show three-point centred moving averages for hormones (—) and I_G (---) series. Missing months were replaced with computed values (linear interpolation of two neighbouring values) before running the three-point centred average function.

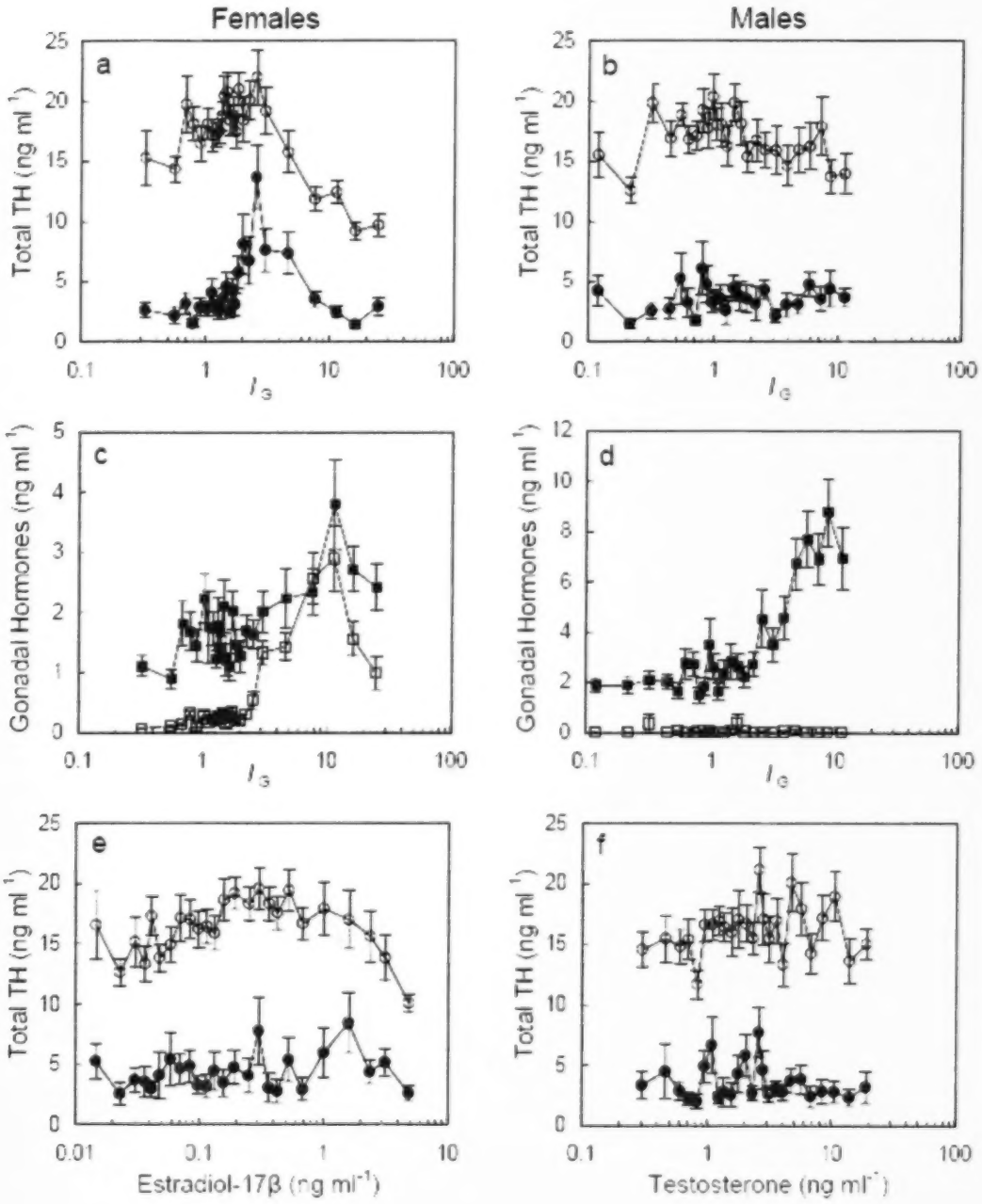

Figure 2. Interrelationships between total thyroid hormones (either total T_4 or total T_3), I_G, and gonadal hormones. Data points represent means from 25 percentile groups categorized based on variable *x*. Data collected throughout the year were included in the analysis. ●, total T_4; ○, total T_3; □, E_2; ■, testosterone. Error bars show S.E.

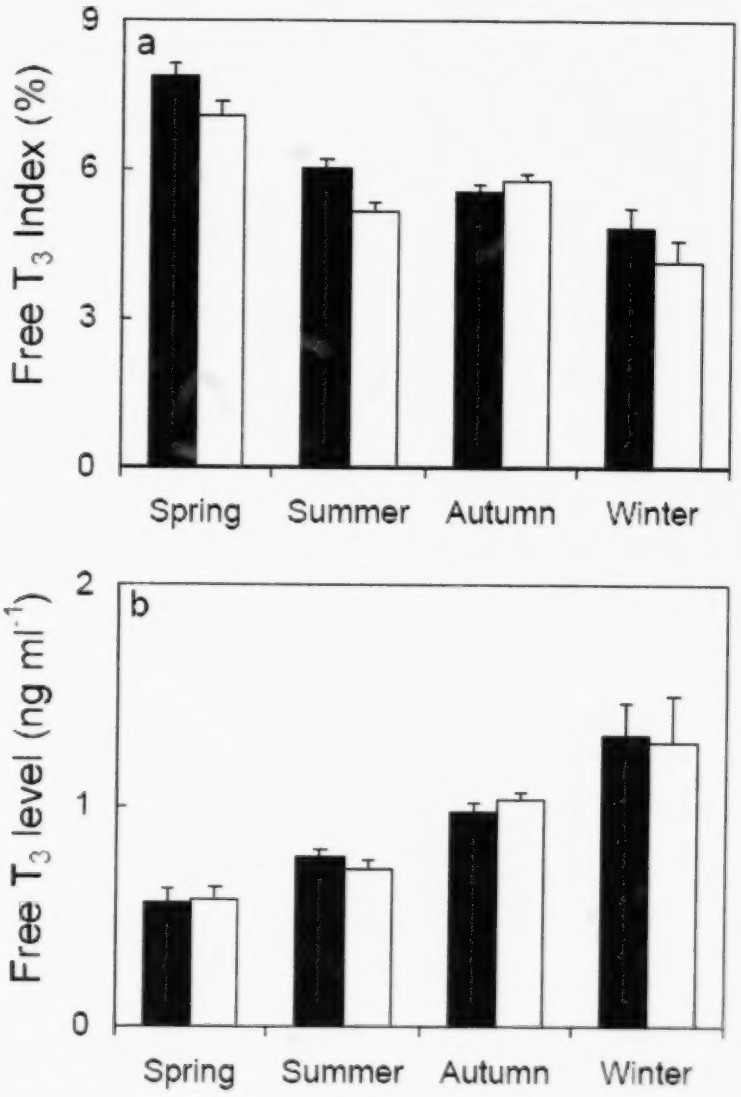

Figure 3. Seasonal averages of the free T_3 index (a) and free T_3 levels (b) in males (filled bars) and females (open bars). Error bars show S.E.

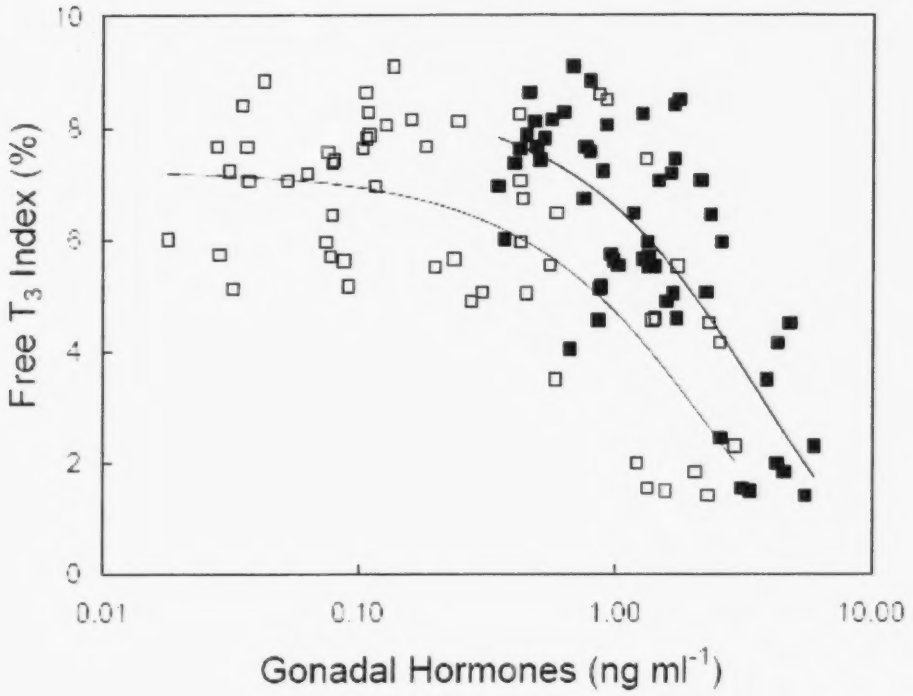

Figure 4. Relationships between the $\log_{10}$ free T_3 index and E_2 (□, $r^2 = 0.51$, $P < 0.001$) in females, and also between the $\log_{10}$ free T_3 index and testosterone (■, $r^2 = 0.60$, $P < 0.001$) in females. Data points represent individual fish sampled between November and January (1996 and 1997).

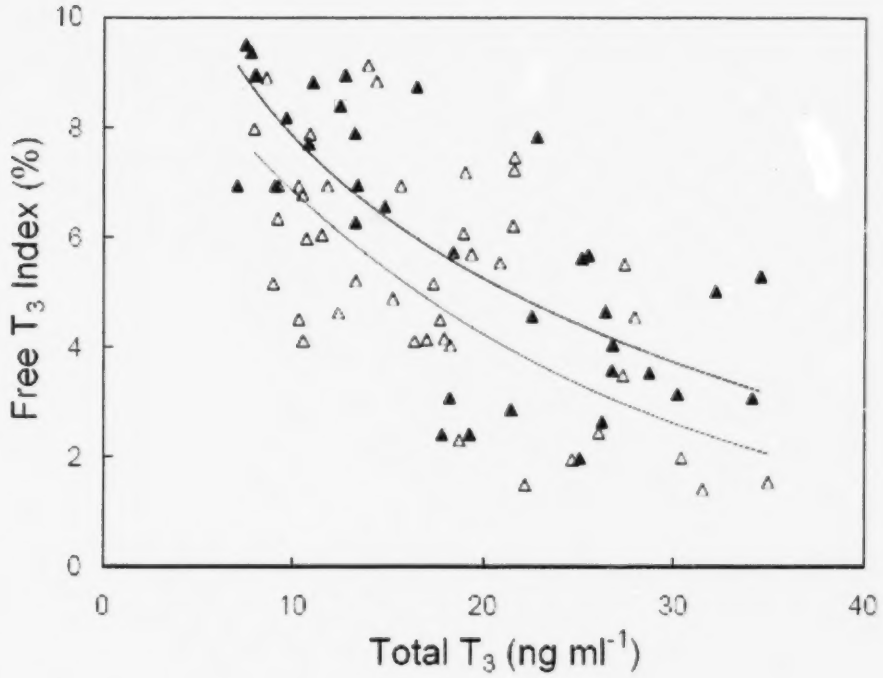

Figure 5. Relationships between the $\log_{10}$ free T_3 index and total T_3 levels in females (Δ, r^2 = 0.45, $P < 0.001$), and also between the free T_3 index and $\log_{10}$ total T_3 levels in males (▲, $r^2 = 0.58$, $P < 0.001$). Data points represent individual fish sampled between January and March (1996 and 1997).